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Early ethanol and water intake: Choice mechanism and total fluid regulation operate in parallel in male alcohol preferring (P) and both Wistar and Sprague Dawley rats

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HIGHLIGHTS

- Initial development of alcohol and water intake in P vs. non-selected rats
- Intake volumes and timing measured precisely, prandial confounds eliminated.
- P rats consume more water when alcohol naïve and more ethanol in forced drinking.
- P vs. non-selected rat EtOH intake in choice is the highest, but total fluid the same.
- Total fluid is the main regulated parameter in choice and a limit to EtOH intake.

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ABSTRACT

The goal of this study was to clarify similar and distinctly different parameters of fluid intake during early phases of ethanol and water choice drinking in alcohol preferring P-rat vs. non-selected Wistar and Sprague Dawley (SD) rats. Precision information on the drinking amounts and timing is needed to analyze micro-behavioral components of the acquisition of ethanol intake and to enable a search for its causal activity patterns within individual CNS circuits. The experiment followed the standard ethanol-drinking test used in P-rat selective breeding, with access to water, then 10% ethanol (10E) as sole fluids, and next to ethanol/water choice. The novelty of the present approach was to eliminate confounding prandial elevations of fluid intake, by time-separating daily food from fluid access. P-rat higher initial intakes of water and 10E as sole fluids suggest adaptations to ethanol-induced dehydration in P vs. Wistar and SD rats. P-rat starting and overall ethanol intake during the choice period were the highest. The absolute extent of ethanol intake elevation during choice period was greatest in Wistar and their final intake levels approached those of P-rat, contrary to the hypothesis that selection would produce the strongest elevation of ethanol intake. The total daily fluid during ethanol/water choice period was strikingly similar between P, Wistar and SD rats. This supports the hypothesis for a universal system that gauges the overall intake volume by titrating and integrating ethanol and water drinking fluctuations, and indicates a stable daily level of total fluid as a main regulated parameter of fluid intake across the three lines in choice conditions. The present findings indicate that a stable daily level of total fluid comprises an independent physiological limit for daily ethanol intake. Ethanol drinking, in turn, stays under the ceiling of this limit, driven by a parallel mechanism of ethanol/water choice.

1. Introduction

In a quest for animal models to study the neurobiological basis of alcoholism, at least seven high alcohol consuming lines of rats were selectively bred over the years [1]. The Indiana University alcohol preferring (P) line, derived from the non-selected (common-stock) Wistar line, has received the most attention [1,2] and meets all criteria [3–5] proposed for an animal model of alcoholism. The P rat choice drinking of alcohol (10% v/v ethanol solution in water, 10E) vs. free water (see Section 2.3) develops over at least three weeks to reach selection criteria of 5 g/kg/day intake and 2:1 preference [6,7]. The existing studies utilize traditional parameters of daily intake and preference to measure choice consumption of ethanol. However, the nature of drinking entangles ethanol consumption with the inevitable intake of water as part of the same solution. Both free water and ethanol solutions together may be subject to regulatory influences governing total daily

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intake of fluid. Moreover, both are influenced strongly by concurrent feeding. There is a need for a strategy to deal with the confounds of feeding imposed modulation and the entangled nature of water and ethanol drinking.

Based on previous reports, certain questions concerning initial differences that may exist in P versus non-selected rats may be evident even prior to the establishment of choice drinking of ethanol: (a) — Are there differences in water intake between ethanol-naïve P and non-selected rats? (b) — Are there differences seen in initial drinking of an ethanol solution when given as the sole fluid? (c) — Are there differences in daily levels of ethanol, water and total fluid early in choice drinking? (d) — How early, and in what precise mode do the differences between P and non-selected rats appear and evolve? (e) — Do these represent evidence for differential self-exposure to the pharmacological effects of ethanol? (f) — Finally, are there clear behavioral similarities, which would indicate integrating regulatory mechanisms that override line differences, during on-going drinking, which may comprise another level of regulation for ethanol intake?

Consumption of water is known to be subject to a variety of influences [11]. Since ethanol is diluted in water, ethanol intake may result either directly through independent choice or in a secondary fashion through other reasons that directly affect water ingestion. For instance, taste, access schedule, and environmental variables can affect drinking. Food access is particularly important, since 70% to 85% of all fluid consumption naturally occurs in close time association with feeding, i.e., within minutes of food intake [12–14] — prandial drinking. Parameters of feeding and associated drinking vary between rats, and carry individual and line related differences [15–17]. Thus, prandial confounds prevent generation of unbiased, fluid-specific drinking sequences. Prandial drinking has been routinely precluded in numerous studies where animals had access only to fluid, including ethanol, or only to food, in experimental sessions lasting up to several hours. Our early tests indicated that the elimination of prandial confounds was necessary over multiple successive days, to make meaningful comparison of fluid intake parameters between lines. To achieve this, we devised a novel strategy of isolating the food from fluid access within each 24-hour cycle. With this approach, fluids are provided in long sessions overlapping the dark phase of a normal lighting cycle. Free food access in the light phase, separated from drinking sessions by 30-min intervals, allows normal weight gain. This design prevented food deprivation that is known to affect drug and ethanol intake, when body weight is deliberately driven down from the free feeding level before an experiment [20,21]. The arrangement provides an optimal compromise to preclude prandial confounds and allow focused analysis of ethanol vs. water consumption between rat lines in choice conditions.

Our long-range goal is to clarify the CNS circuits and signals that mediate drinking of ethanol and ways of modulating such signals to control its intake. Accordingly, an additional motivation for this work was to gain knowledge on ethanol intake with a very high level of temporal resolution to relate, at a later stage, on-going drinking behavior to causal spatial–temporal patterns of single-neuron activity within specific circuits of the mesolimbic system. An extended effort to develop new instrumentation and evolve the protocol has been presented in preliminary reports [8–10]. Drinking was studied in precise detail using new lick-sensing spouts and software, in lab cages optimized for concurrent recording of neural activity in free-behaving rats.

The aim of the present study was to characterize early drinking of ethanol, water and total fluid by P rat, with Wistar and Sprague Dawley (SD) non-selected albino rats serving as comparison lines. Wistar is the parental line of the P rat whereas SD is a non-related line, yet both have shown previously similar levels of ethanol intake under certain conditions [18,19]. Our protocol followed the general timeline of a drinking test used for P-rat selective breeding. Initially water, and then 10E, was available as the sole fluid. Last, 10E and water were available concurrently. The instrumentation yielded an integrated body of information on daily intake as well as temporal parameters of drinking ethanol and water. The analysis of drinking patterns and parameters of ethanol and water bouts in P, Wistar and SD rats are presented in our concurrent report. Here we present data on daily intake in the three lines of rats across the phases of the experiment. A preliminary hypothesis was that selective breeding results in consistently higher ethanol intake even during early access to ethanol, while combined levels of ethanol solution and water intake in choice conditions may be subject to a universal systemic regulation of total fluid intake.

2. Material and methods

2.1. Animals

Adult male P rats (59th–60th generation, n = 10, Indiana U., IN) weighed 200 g, and Wistar (n = 8) and Sprague Dawley (SD, n = 14) rats (both from Harlan, IN) weighed 325–350 g upon arrival. P rats were double-housed until growing to 325–350 g, and from this weight all animals were single-housed, in filter-ventilated cages (Thoren Caging System, Inc., Hazleton, PA) at 22 ± 1.5 °C and 42 ± 8% humidity under normal lighting cycle ("OFF" = 18:00, "ON" = 06:00) with food and water ad-lib. Body weights were measured once a week until the start of experiment. All animals entered the study upon reaching the weight of 400 g. Experimental procedures were approved by the Institutional ACUC and are in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

Plexiglas cages measured 40 × 35 × 35 cm (L × W × H) and were located in separate light-insulated, sound-attenuated and individually ventilated cubicles. Each cage contained a house light and a fan on top and two spouts, located symmetrically on the sidewalls, 6 cm from the back corners, and 4 cm above the floor. Spouts, custom made in the laboratory, required insertion of the tongue down into a spout well to obtain fluid. Lick movements were monitored by an infrared beam-photosensor setup. Fluids were delivered into the spouts at a rate of 90.25 μl/s with model R-E syringe pumps (Razel Scientific Instruments, St. Albans, VT). The data collection and instrumentation control software (Neuroplex, Inc., Winston-Salem, NC) provided monitoring, execution and recording of each lick and fluid delivery at 1 ms resolution.

2.3. Experimental design

The experiment consisted of three test phases (periods). (1) — Water phase: access to 100% tap water alone, as the sole fluid, with no additives (free water, water), for 5 days. (2) — Ethanol phase: access to 10% v/v solution of ethanol in tap water (10E) alone, as the sole fluid, for 5 days. (3) — Choice period, also called 'ethanol/water choice': access to concurrent 10E and free water for 12 days. In sole fluid conditions, on day 1 fluid was accessible at both sides, on day 2 at the left side only, on day 3 at the right side only, and so on. In choice conditions, on day 1 water was accessible at the left side and 10E at the right, on day 2 access sides were reversed and fluid presentation continued with daily side alternation. Phases of experiment followed each other with no interruptions, each animal passed through all 22 sessions.

2.4. Procedures

2.4.1. Adaptation

Before the experiment, animals went into experimental cages for 8-h exposure on each of three successive days. In these pre-runs, animals stayed in experimental cages from 08:30 to 16:30, with isolating cubicles open, lights in the experimental room "ON" and ad-lib food placed on the cage floor but no fluid access and no data collection. Between and after the pre-runs animals stayed in their home-cages.